abcam

Product datasheet

Anti-S6K1 antibody [E343] ab32529

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-S6K1 antibody [E343]
Description	Rabbit monoclonal [E343] to S6K1
Host species	Rabbit
Specificity	This antibody detects both alpha I and alpha II isoforms.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human S6K1 aa 1-100 (N terminal). The exact sequence is proprietary.
Positive control	WB: WT HAPI, MCF7 and HEK293 cell lysates. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa, 293T, Neuro-2a and C6 cells. IHC-P Human breast cancer, mouse testis and rat brain tissue.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E343
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32529 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/100 - 1/2200. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.
WB		1/5000 - 1/10000. Detects a band of approximately 70 kDa (predicted molecular weight: 59 kDa). For Rat and Mouse samples 1/500 dilution has only been tried. We have not tested if similarly to Human samples a lot higher dilutions can be used.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/110.

Target

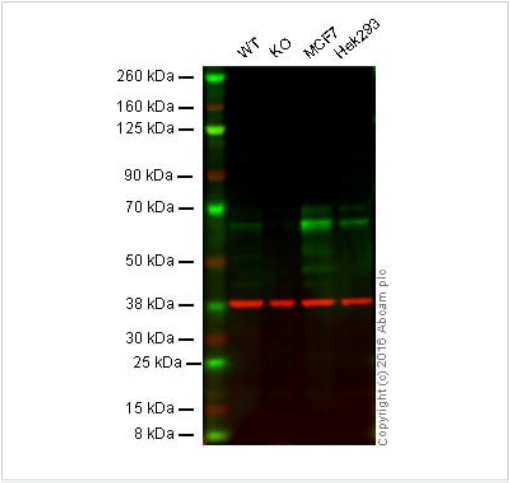
Function Acts to integrate nutrient and growth factor signals in regulation of protein synthesis, cell proliferation, cell growth, cell cycle progression and cell survival. Downstream effector of the mTOR signaling pathway. Phosphorylates specifically ribosomal protein S6 in response to insulin or several classes of mitogens. During translation initiation, the inactive form associatess with the eIF-3 complex under conditions of nutrient depletion. Mitogenic stimulation leads to phosphorylation and dissociation from the eIF-3 complex and the free activated form can phosphorylate other translational targets including EIF4B. Promotes protein synthesis by phosphorylating PDCD4 at 'Ser-67' and targeting it for degradation. Phosphorylates RICTOR leading to regulation of mammalian target of rapamycin complex 2 (mTORC2) signaling; probably phosphorylates RICTOR at 'Thr-1135'. Phosphorylates IRS1 at multiple serine residues coupled with insulin resistance; probably phosphorylates IRS1 at 'Ser-270'. Required for TNF-alpha induced IRS-1 degradation. Phosphorylates EEF2K in response to IGF1 and inhibits EEF2K activity. Phosphorylates BAD at 'Ser-99' in response to IGF1 leading to BAD inactivation and inhibition of BAD-induced apoptosis. Phosphorylates mitochondrial RMP leading to dissociation of a RMP:PPP1CC complex; probably phosphorylates RMP at 'Ser-99'. The free mitochondrial PPP1CC can dephosphorylate RPS6KB1 at Thr-412 which is proposed to be a negative feed back mechanism for the RPS6KB1 antiapoptotic function. Phosphorylates GSK3B at 'Ser-9' under conditions leading to loss of the TSC1-TSC2 complex. Phosphorylates POLDIP3.

Tissue specificity Widely expressed.

Sequence similarities Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. S6 kinase subfamily.
Contains 1 AGC-kinase C-terminal domain.
Contains 1 protein kinase domain.

Domain	The autoinhibitory domain is believed to block phosphorylation within the AGC-kinase C-terminal domain and the activation loop. The TOS (TOR signaling) motif is essential for activation by mTORC1.
Post-translational modifications	Phosphorylation at Thr-412 is regulated by mTORC1. The phosphorylation at this site is maintained by an agonist-dependent autophosphorylation mechanism.
Cellular localization	Cytoplasm; Nucleus. Cytoplasm and Cell junction > synapse > synaptosome. Mitochondrion outer membrane.

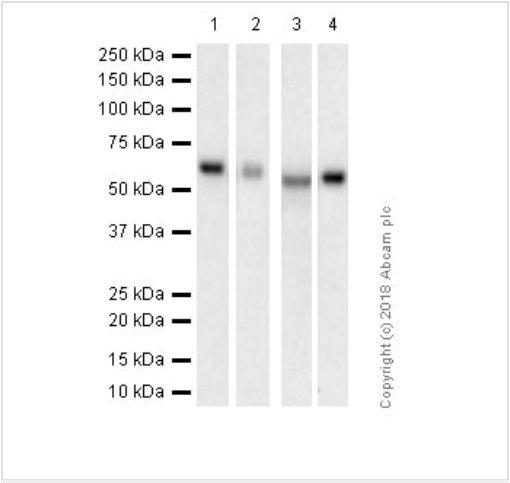
Images



Western blot - Anti-S6K1 antibody [E343] (ab32529)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: S6K1 knockout HAP1 cell lysate (20 µg)
Lane 3: MCF7 cell lysate (20 µg)
Lane 4: HEK293 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab32529 observed at 68 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab32529 was shown to recognize S6K1 when S6K1 knockout samples were used, along with additional cross-reactive bands. Wild-type and S6K1 knockout samples were subjected to SDS-PAGE. ab32529 and **ab8245** (loading control to GAPDH) were diluted 1/5000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-S6K1 antibody [E343] (ab32529)

All lanes : Anti-S6K1 antibody [E343] (ab32529) at 0.004 µg/ml (purified)

Lane 1 : Neuro2a (Mouse neuroblastoma neuroblast) whole cell lysate
Lane 2 : Mouse cerebellum lysate
Lane 3 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 4 : Rat cerebellum

Lysates/proteins at 20 µg per lane.

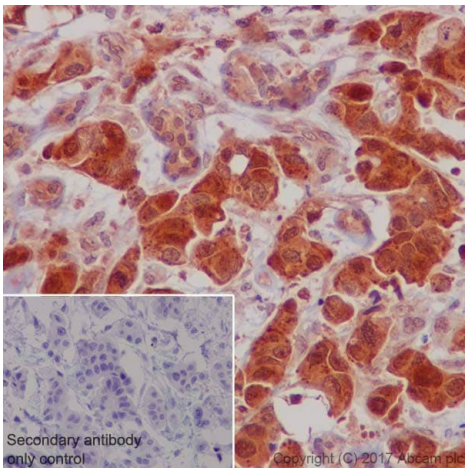
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 59 kDa

Observed band size: 70 kDa

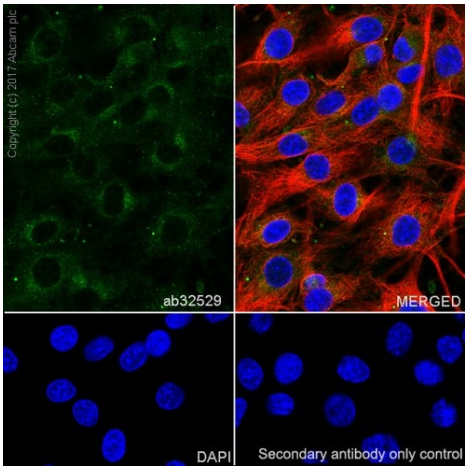
Exposure time: 3 minutes

Blocking and diluting buffer used: 5% NFDM/TBST.



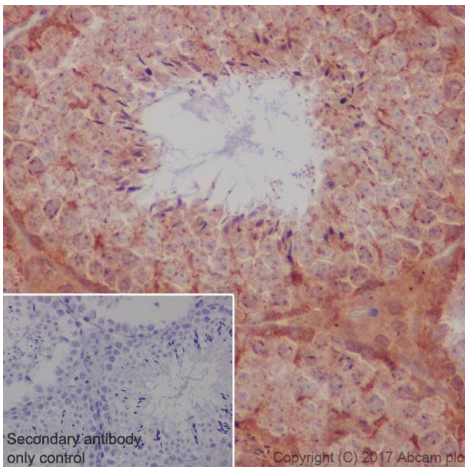
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S6K1 antibody [E343] (ab32529)

Immunohistochemical analysis of Human breast cancer tissue labeling S6K1 with ab32529 at 1/500 dilution (4.4 µg/mL). The secondary antibody used was ImmunoHistoProbe one step HRP Polymer (ready to use). Secondary antibody only control-PBS instead of the primary antibody. Antigen retrieval was heat mediated using **ab93684** (Tris/EDTA buffer, pH 9.0). The tissue was counterstained with Hematoxylin.



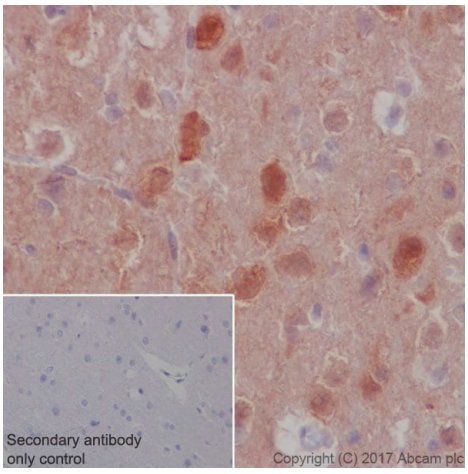
Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] (ab32529)

Immunocytochemistry/Immunofluorescence analysis of C6 cells (Rat glial tumor glial cell) labelling S6K1 with ab32529 at a dilution of 1:200, 11.1 µg/ml. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. A 1:1000 dilution (2µg/ml) was used for the secondary antibody Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**). The cells were co-stained with 1:200, 2.5µg/ml with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594). Nuclei counterstained with DAPI (blue). Control: 1:1000 dilution.



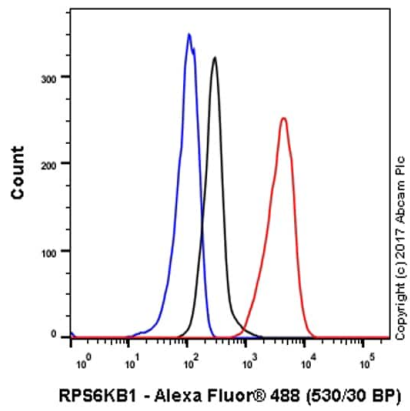
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S6K1 antibody [E343] (ab32529)

Immunohistochemical analysis of mouse testis tissue labeling S6K1 with ab32529 at 1/500 dilution (4.4 µg/mL). The secondary antibody used was ImmunoHistoProbe one step HRP Polymer (ready to use). Secondary antibody only control-PBS instead of the primary antibody. Antigen retrieval was heat mediated using **ab93684** (Tris/EDTA buffer, pH 9.0). The tissue was counterstained with Hematoxylin.



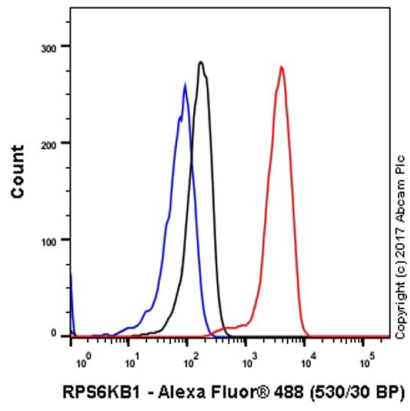
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S6K1 antibody [E343] (ab32529)

Immunohistochemical analysis of rat brain tissue labeling S6K1 with ab32529 at 1/500 dilution (4.4 µg/mL). The secondary antibody used was ImmunoHistoProbe one step HRP Polymer (ready to use). Secondary antibody only control-PBS instead of the primary antibody. Antigen retrieval was heat mediated using **ab93684** (Tris/EDTA buffer, pH 9.0). The tissue was counterstained with Hematoxylin.



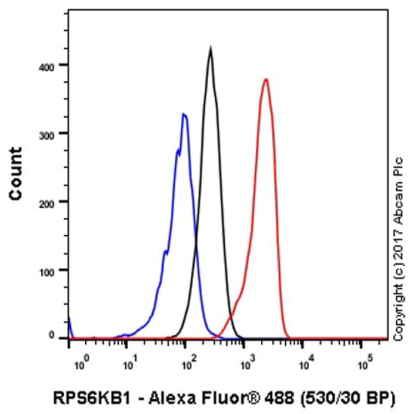
Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] (ab32529)

Intracellular Flow Cytometry analysis of Neuro-2a (Mouse neuroblastoma neuroblast) cells labelling with ab32529 (purified) at 1/2200 dilution (1µg/mL) (red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluorr® 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution. Isotype control - 90% methanol . Unlabeled control - Rabbit monoclonal IgG (**ab172730**) / Black.



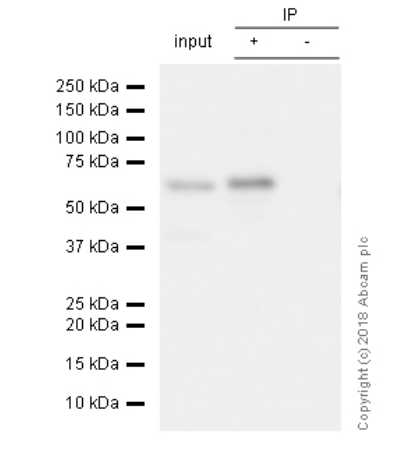
Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] (ab32529)

Intracellular Flow Cytometry analysis of 293T (Human embryonic kidney epithelial cell) cells labelling with ab32529 (purified) at 1/2200 dilution (1µg/mL) (red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluorr® 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution. Isotype control - 90% methanol . Unlabeled control - Rabbit monoclonal IgG (**ab172730**) / Black.



Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] (ab32529)

Intracellular Flow Cytometry analysis of C6 (Rat glial tumor glial cell) cells labelling with ab32529 (purified) at 1/2200 dilution (1µg/mL) (red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluorr® 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution. Isotype control - 90% methanol . Unlabeled control - Rabbit monoclonal IgG (**ab172730**) / Black.

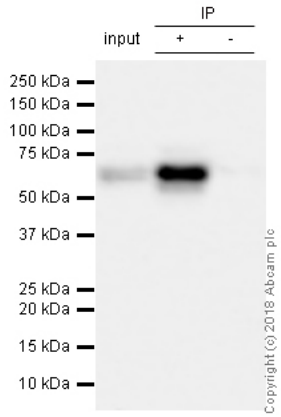


Immunoprecipitation - Anti-S6K1 antibody [E343] (ab32529)

Lane 1: Neuro2a (Mouse neuroblastoma neuroblast) whole cell lysate, 10µg
Lane 2: Neuro2a whole cell lysate 350µg and ab32529, 2µg
Lane 3: Neuro2a cell lysate, 350µg and rabbit IgG (**ab172730**), 2µg

Purified ab32529 immunoprecipitating S6K1 in HEK293T cell lysates. Primary antibody was used at a 1/110 dilution (20 µg/ml). For western blotting, ab32529 at 1/500 and VeriBlot for IP (HRP) **ab131366** was used for detection at 1/1000 dilution.

Blocking and diluting buffer used: 5% NFDM/TBST.

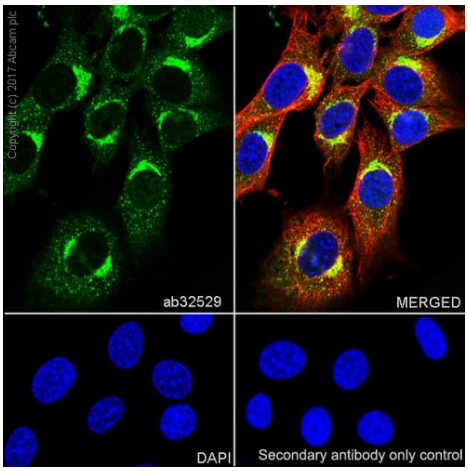


Immunoprecipitation - Anti-S6K1 antibody [E343] (ab32529)

Lane 1: HEK293T (Human embryonic kidney epithelial cell) whole cell lysate, 10µg
Lane 2: HEK293T whole cell lysate, 10µg and ab32529, 2µg
Lane 3: HEK293T cell lysate, 350µg and rabbit IgG (**ab172730**) , 2µg

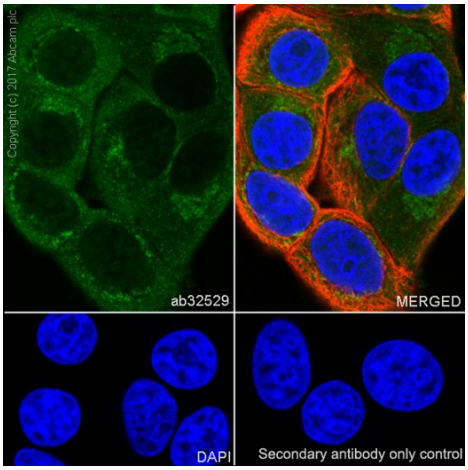
Purified ab32529 immunoprecipitating S6K1 in HEK293T cell lysates. Primary antibody was used at a 1/110 dilution (20 µg/ml). For western blotting, ab32529 at 1/500 and VeriBlot for IP (HRP) **ab131366** was used for detection at 1/1000 dilution.

Blocking and diluting buffer used: 5% NFDM/TBST.



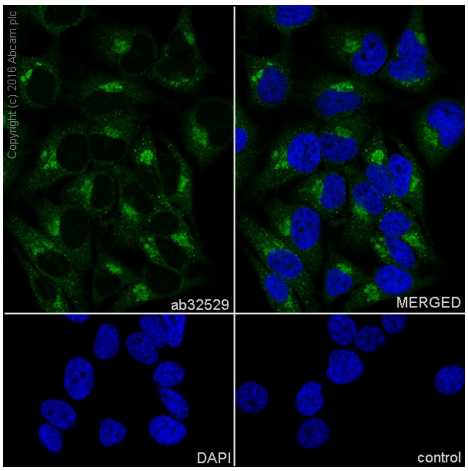
Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] (ab32529)

Immunocytochemistry/Immunofluorescence analysis of NIH/3T3 (Mouse embryonic fibroblast) labelling with ab32529 at a dilution of 1:200, 11.1 µg/ml. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. A 1:1000 dilution (2µg/ml) was used for the secondary antibody Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**). The cells were co-stained at 1:200 dilution, 2.5µg/ml with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594). Nuclei counterstained with DAPI (blue). Control: 1:1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] (ab32529)

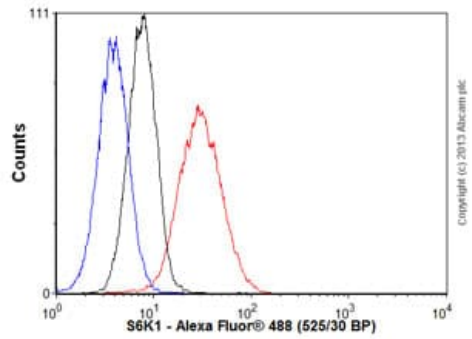
Immunocytochemistry/Immunofluorescence analysis of MCF 7 (Human breast adenocarcinoma epithelial cell) labeling S6K1 with ab32529 at a dilution of 1:200, 11.1 ug/ml. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. A dilution of 1/1000 (2µg/ml) was used for the secondary antibodyGoat anti rabbit IgG (Alexa Fluor® 488, **ab150077**). The cells were co-stained at 1:200 dilution, 2.5µg/ml with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) . Nuclei counterstained with DAPI (blue). Control: 1:1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-S6K1 antibody [E343] (ab32529)

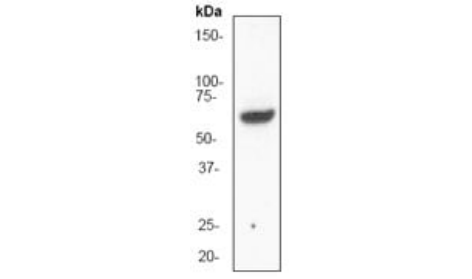
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling S6K1 with ab32529 at a dilution of 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. **ab150077** at 1/1000 was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).

Confocal image showing cytoplamic staining on HeLa cell line.



Flow Cytometry (Intracellular) - Anti-S6K1 antibody [E343] (ab32529)

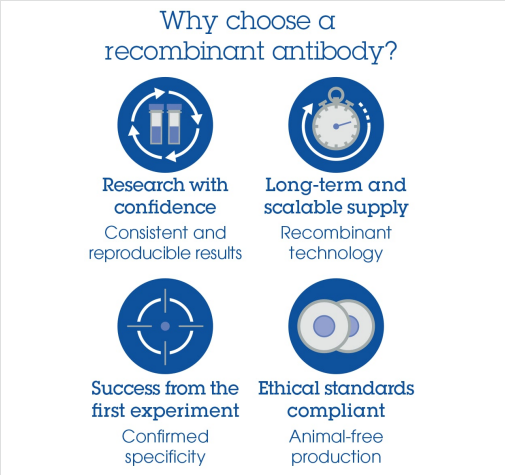
Overlay histogram showing HeLa cells stained with ab32529 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32529, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Western blot - Anti-S6K1 antibody [E343] (ab32529)

Anti-S6K1 antibody [E343] (ab32529) at 1/10000 dilution + 293T cell lysate

Predicted band size: 59 kDa
Observed band size: 70 kDa



Anti-S6K1 antibody [E343] (ab32529)

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